SANTA CRUZ BIOTECHNOLOGY, INC.

MTHFD2 siRNA (h): sc-75937

BACKGROUND

MTHFD2 (methylenetetrahydrofolate dehydrogenase 2), also known as NMDMC, is a 350 amino acid bifunctional protein that is responsible for the consecutive interconversion of tetrahydrofolate derivatives which drive the synthesis of purine, methionine and thymidylate. MTHFD2 is bifunctional in that it has methylenetetrahydrofolate dehydrogenase and methenyltetrahydrofolate cyclohydrolase activity. MTHFD2 requires either NADP or NAD as a cofactor for interconversion. Activity of these cofactors is affected by intracellular magnesium and phosphate concentrations. MTHFD2 functions as a homodimer and is localized to the mitochondria where it is expressed during the development of normal tissue.

REFERENCES

1. Shannon, K.W., et al. 1986. Purification and characterization of a mitochondrial isozyme of C1-tetrahydrofolate synthase from *Saccharomyces cerevisiae*. J. Biol. Chem. 261: 12266-12271.
2. Peri, K.G., et al. 1989. Nucleotide sequence of the human NAD-dependent methylene tetrahydrofolate dehydrogenase-cyclohydrolase. Nucleic Acids Res. 17: 8853.
3. Schild, D., et al. 1990. Cloning of three human multifunctional *de novo* purine biosynthetic genes by functional complementation of yeast mutations. Proc. Natl. Acad. Sci. USA 87: 2916-2920.
4. Yang, X.M., et al. 1993. NAD-dependent methylenetetrahydrofolate dehydrogenase-methenyltetrahydrofolate cyclohydrolase is the mammalian homolog of the mitochondrial enzyme encoded by the yeast MIS1 gene. Biochemistry 32: 11118-11123.

CHROMOSOMAL LOCATION

Genetic locus: MTHFD2 (human) mapping to 2p13.1.

PRODUCT

MTHFD2 siRNA (h) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see MTHFD2 shRNA Plasmid (h): sc-75937-SH and MTHFD2 shRNA (h) Lentiviral Particles: sc-75937-V as alternate gene silencing products.

For independent verification of MTHFD2 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-75937A, sc-75937B and sc-75937C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

MTHFD2 siRNA (h) is recommended for the inhibition of MTHFD2 expression in human cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 µM in 66 µl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

MTHFD2 (A-2): sc-390708 is recommended as a control antibody for monitoring of MTHFD2 gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor MTHFD2 gene expression knockdown using RT-PCR Primer: MTHFD2 (h)-PR: sc-75937-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Shao, C., et al. 2020. Cytosolic ME1 integrated with mitochondrial IDH2 supports tumor growth and metastasis. Redox Biol. 36: 101685.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.